REMARKS

Introduction

Receipt is acknowledged of a non-final office action dated June 25, 2003. In the action, the examiner objected to claims 2, 4, 5, 19 and 52 for containing non-elected subject matter and rejected claims 1-8, 10-12, 14, 19, 20, 23, 33 and 34 for allegedly failing to meet the written description requirements, claims 1-8, 10-12, 14, 19, 20, 23, 33, 34 and 52 for alleged non-enablement, and claims 2-4, 5, 20 and 34 for alleged indefiniteness. Additionally, the examiner rejected claims 1-5, 7, 8, 10-12, 14, 19, 20, 33 and 34 for alleged lack of novelty, and claims 6, 23 and 52 for obviousness reasons.

Status of the Claims

In this response, applicants amended claims 1, 2, 4-8, 10-12, 14, 19, 23, 33, 50 and 52 to read on elected subject matter and more clearly define the present invention. Support for amended claim 1 can be found throughout the specification. See, for example, page 1, lines 1-3.

Applicants also added new claims 62-64, and cancelled claims 3, 20 and 34. Support for new claims 62-64 can be found in originally filed claims 1, 7 and 2, respectively. Upon entry of this amendment, claims 1-2, 4-8, 10-12, 14, 19, 23, 33, 50, 52 and 62-64 will be under examination.

Drawings

The examiner objected to the drawings for essentially being of poor quality. Submitted herewith are formal drawings that address the examiner's objections.

Priority

Applicants submit herewith a certified copy of an application filed in France on October 3, 2000, which is applicants' priority document.

Claim Objections

The examiner objected to claims 2, 4, 5, 19 and 52 for containing non-elected subject matter, and claim 10 for being dependent upon a non-elected claim. Accordingly, applicants amended claims 2, 4, 5, 19 and 52 so as to only recite the elected inventions.

Applicants, however, reserve the right to file divisional applications covering non-elected subject matter.

Regarding claim 10, which depends on claim 8, is under consideration in the present application and therefore, applicants are unclear as to basis for the examiner's objection.

35 U.S.C. 112, first paragraph

Written Description

The examiner rejected claims 1-8, 10-12, 14, 19, 20, 23, 33 and 34 for allegedly failing to meet the written description requirement. In particular, the examiner asserted that while "[t]he claims encompass any type of metazoan organism comprising in at least one cell...a fusion protein comprising a recombinase, a hinge region, and a polypeptide comprising the ligand binding domain of the human nuclear estrogen receptor;...[and] one or more gene[s] of interest flanked by recognition sites of said recombinase," the "specification only describes a transgenic mouse comprising claimed elements" (office action at 4-5).

Applicants respectfully disagree. "An objective standard for determining compliance with the written description requirement is, 'does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed'" (M.P.E.P. § 2163.02, citing *In re Gostelli*, 872 F.2d 1008, 1012 (Fed. Cir. 1989)). Beginning on page 8, first full paragraph of the specification, a "metazoan organism" is defined.

Nevertheless, in the interest of expediting prosecution, and without acquiescing to the examiner's rejection, applicants amended claim 1 to recite a transgenic mouse.

The examiner also rejected the claims for reciting natural variants or fragments of a human or vertebrate nuclear estrogen receptor. In other words, the examiner believes that "the specification only discloses a fusion protein comprising Cre and [a] ligand binding domain of [a] human nuclear estrogen receptor" and that fragments or variants of the estrogen receptor are not described (office action at 5).

Likewise, the examiner stated that a natural or synthetic variant of the claimed recombinase fails to meet the written description requirement (*id.*). However, one of skill in the art would know what is meant by a natural or synthetic recombinase, and in fact, the

specification describes variants of the recombinase protein (see, specification at 10, lines 8-25). The term "natural variant" as recited in the claims is also defined on page 13, lines 1-10, and a definition of "nuclear receptor fragment" can be found on page 13, lines 19-21. In addition, a fragment can be easily identified by an assay measuring LBD activity.

Enablement

Continuing, the examiner rejected claims 1-8, 10-12, 14, 19, 20, 23, 33, 34 and 52 for alleged non-enablement, asserting that the specification is "enabling for a transgenic mouse comprising a first transgene comprising Cre recombinase fused to a mutated ER, wherein such mutation [would] result in conditional activation of Cre upon synthetic ligand treatment...[and] a second transgene comprising insertion Cre recognition sites loxP flanking the gene of interest...[the specification] does not reasonably provide enablement for any metazoan organism comprising a cell comprising claimed transgenes" (office action at 6).

As discussed above, applicants amended the claims so as to read on a transgenic mouse. Additionally, specific working examples are directed to targeted inactivation of the RXRα gene in the epidermis of adult mice (example 1), the SNF2β gene in the epidermis of adult mice (example 2), the RXRα gene in murine adipocytes, the RXRα gene in murine hepatocytes, sensitivity of Cre-ER^{T3} and Cre-ER^{T2} to tamoxifen, and role of RXRα in skin carcinogenesis. Thus, the specification teaches stage and tissue-specific modification in three cell populations (keratinocytes, adipocytes and hepatocytes) of three distinct organs using different transgenic mice expression fusion proteins.

35 U.S.C. 112, second paragraph

The examiner also rejected claims 2-4, 5, 20 and 34 allegedly for indefiniteness. Specifically, the examiner stated that the phrase "of the bacterial β recombinase" is unclear (claims 2-3), that there is insufficient antecedent basis for "said" Cre recombinase (claims 4 and 5) and that it is unclear whether the limitations following the phrase "in particular" are part of the claimed invention (claims 20 and 34).

Claim 2 has been amended to recite the elected invention, thereby rendering this rejection moot.

Additionally, applicants amended claim 4 to recite "said recombinase protein" to be consistent with the wording in claim 1. However, with regard to claim 5, applicants

respectfully assert that the phrase "said Cre recombinase" is not a recited limitation in the claim. As such, rejection of claim 5 is improper.

Claims 20 and 34 have been cancelled, thereby rendering this rejection moot.

35 U.S.C. 102

The examiner rejected claims 1-5, 7, 8, 10-12, 14, 20, 33 and 34 under 35 U.S.C. § 102(a) as allegedly being anticipated by Indra *et al.*, *Nucleic Acids Res.*, 27:4324 (1999).

Applicants respectfully assert that Indra is not prior art since the reference only teaches an exogenous reporter gene (CAT gene) that was introduced beforehand into a host chromosome. Indra, as well as Feil *et al.* (see below) show that treating mice that express Cre-ER^{T} or Cre-ER^{T2} with tamoxifen or hydroxytamoxifin will induce the deletion of segments of DNA that have been previously inserted into the genome by transgenesis and that carry selection markers or reporter genes framed by LoxP sites. The present invention, however, teaches endogenous DNA sequences in their normal chromatin environment.

Continuing, the examiner rejected claims 1-5, 7, 8, 10-11, 19, 20, 33 and 34 under 35 U.S.C. § 102(b) as allegedly being anticipated by Feil *et al.*, *PNAS*, 26:1427 (1998). Specifically, the examiner stated that Feil "disclose[d] the generation of a double transgenic mouse comprising a reporter cassette that comprises tkneo selection marker flanked by two lox P sites that integrated into RXRα allele, and another casette comprising Cre-ER^{T} under the control of CMV promoter" and further, that "OHT administration resulted [in] Cre mediated excision of [a] RXRα gene" (office action at 13).

Applicants respectfully assert achieving tightly temporally controlled somatic mutations in the mouse, targeted to endogenous genes in their normal chromosomal position and environment, was not anticipated by published work on Cre-ER fusion proteins at the time of filing. For example, Feil fail to teach an essential feature of the present invention, i.e., that the chromosomal gene of interest, which is the target of excision, is endogenous to the mouse. In other words, Feil describe the excision, controlled in both time and space, of exogenous reporter genes that were introduced into the host chromosome.

Since Feil and Indra only demonstrate that Cre-ER^{T} or Cre-ER^{T2} fusion proteins, respectively, could be used to delete DNA segments with synthetic reporter

transgenes, neither Feil nor Indra disclose each and every element of the claimed invention. Thus, the present invention is not anticipated.

35 U.S.C. 103

The examiner rejected claims 23 and 52 as allegedly obvious over Indra and Feil, in view of Ross *et al.* (*PNAS*, 87:9590 (1990)) and Tontonoz *et al.* (*PNAS*, 94:237 (1997)). In particular, the examiner asserted that "[i]t would have been obvious to one of ordinary skill of art to make a transgenic mouse with selective RXRα disruption in adipose tissue based on the combined teaching of Indra et al. and Feil et al., Ross et al. and Tontonoz et al." (office action at 14). Additionally, the examiner rejected claim 6 for obviousness reasons, citing Feil, in view of Schwenk *et al.* (*Nucleic Acids Res.*, 26:1427 (1998)).

Applicants respectfully disagree. In order to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art references (or references when combined) must teach or suggest all the claim limitations. See MPEP 2142. Applicants contend that the examiner has not made a showing of obviousness.

The instant invention provides a general method for efficient stage- and tissue-specific modification of a given gene in the living mouse. At the time of filing applicants provided examples for such modifications in three cell populations (keratinocytes, adipocytes and hepatocytes) of three distinct organs using different transgenic mice expressing fusion proteins. Each of the fusion proteins contain a modified estrogen receptor ligand binding domain (ER^T, ER^{T2}) conferring in vivo tamoxifen-inducibility to the activity of the fused Cre recombinase. Transgenic lines were then bred with mice in which endogenous genes, in their normal chromosomal position and environment, contain engineered LoxP sites specifically recognized by a Cre recombinase. In the progeny, the fused Cre recombinase is activated upon tamoxifen treatment and specifically deletes gene segments flanked by LoxP-sites. In each case, the deletion was 100% efficient in all cells in which the recombinase was expressed. Moreover, a deletion was not observed in the absence of tamoxifen treatment, indicating that the inventive method permits tight temporal control of the generation of cell type / tissue-specific somatic mutations.

Thus, achieving tightly temporally-controlled somatic mutations in the mouse, targeted to endogenous genes in their normal chromosomal position and environment, was not obvious from published work on Cre-ER fusion proteins at the time of filing. Likewise, it was not obvious at the time of filing that the present invention would work in any type of cell, whether or not it has the ability to divide or is specialized.

As discussed above, Feil *et al.* (*Proc. Natl. Acad. Sci. USA* 98, 10887-10890, 1996) and Indra *et al.* (Nucl. Acid. Res. 27, 4324-4327, 1999) had **only** demonstrated that Cre-ER^{T} or Cre-ER^{T2} fusion proteins could be used to delete DNA segments within synthetic reporter transgenes.

Additionally, Schwenk *et al.* (*Nucl. Acid. Res.* 26, 1427-1432, 1998), demonstrated that a tamoxifen-inducible Cre-ER fusion protein could be used to delete a chromosomal DNA segment but the efficiency of recombination in cells expressing the fusion protein was variable and never complete. High doses of tamoxifen had to be used to reach 80% deletion, under conditions where the tamoxifen anti-oestrogenic activity become harmful for the mouse. Thus, the tamoxifen-activated Cre-ER fusion protein of Schwenck could not be used to delete a chromosomal DNA segment with an efficiency to validly study gene function.

Tontonoz *et al.* demonstrates that RXR-specific retinoid ligands induce terminal differentiation of human liposarcoma cells. However, Tontonoz does not relate to the technical field of the claimed invention, nor relate to the subject matter thereof. The claimed invention concerns a transgenic mouse, endogenous genes of which can be efficiently excised in both a stage and tissue-specific manner upon tamoxifen treatment. As such, Tontonoz, in view of Indra, Feil and Ross, do not render the claimed invention obvious.

CONCLUSION

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and arguments.

It is respectfully urged that the present application is now in condition for allowance. Early notice to that effect is earnestly solicited.

The examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

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